below are essentially those presented in the first Rule 116 amendment. A Notice of Appeal was mailed July 21, 1994 in order to stop the running of time from the Final Rejection.

The specification has been amended on page 33 to correct a typographical error.

As requested in the Final Rejection, each Figure has been amended to identify units on the y-axis and to provide descriptive indications on the x-axis. A separate letter to the Official Draftsman has been sent with the corrected drawings in proper form. Approval of the corrected drawings is requested.

Claim 38 has been amended a second time as is discussed in more detail below in response to the Final rejection.

New Claims 39-42 are submitted as more narrow embodiments of original Claims 1, 12, 19 and 22. They incorporate the features of canceled Claims 9, 15, 32 and 34 into the independent claims. Applicants respectfully request entry of these amendments in their sincere attempt to expedite prosecution. No new issue is raised and no new matter is presented since the incorporated language is taken from the canceled claims which have already been searched. It is also believed that these new claims are more readily distinguishable over the cited prior art.

Rejection Under 35 U.S.C. 112, Second Paragraph

Claims 1-8 and 10-38 have been rejected as being indefinite for failing to recite specific oligonucleotide sequences. As far as it applies to claims presently in this application, this rejection is respectfully traversed.

Applicants arguments, presented in their earlier response, are repeated here for Claims 1-8, 10-14, 16-31, 33 and 35-37 because those arguments remain cogent. The Final Rejection is considered to be seeking to limit all claims to specific oligonucleotide sequences when the Statute does not require it. Clearly, generic compounds have been patented in the past in various

technologies using functional limitations and features without the need for specific chemical compositional formulae. Clearly, Applicants intend to claim every sequence that falls within the claims parameters because such relationships have not been described in the art for amplification compositions, kits and methods. Applicants clearly indicate in their specification how such primers can be chosen and used, and that is sufficient to make the metes and bounds of the claims understandable to those skilled in the art, which even the Final Rejection alleges, are doctoral scientists. Clearly, such educated people can understand from Applicants' claims and specification how to choose matched primers for use in the claimed invention for any given target nucleic acid sequence.

The Final Rejection has objected to the functional nature of the recitation of oligonucleotides, and supports its arguments by reference to the treatment of chemical compound inventions by the P.T.O.B.P.I. in *Ex parte D.*

Granted that the Board indicates in the noted case that DNA sequences are complex chemical compounds. However, this case is irrelevant to the issue at hand. It relates to a prior art rejection of a compound *per se*, not to aqueous solutions, test kits and assay methods, as in the present application. In the only compound claim of this application, Claim 38, Applicants have recited the nucleotide sequences.

Similar to other cases involving "chemical compounds", Applicants are allowed by the Statute to recite the use of chemical compounds in functional terms, i.e. by what the primers and probes do, not by their structure, *In re Fuetterer* 138 U.S.P.Q. 217 (C.C.P.A., 1963). This case remains good law and has been followed for decades by the Board and C.A.F.C. The Examiner is urged to follow this case law. The metes and bounds of Applicants claimed invention are well within the routine experimentation of a skilled artisan because Applicants have provided sufficient teaching in their specification

as to how to choose appropriate primers and probes (pages 11-16). Other knowledge in the art would readily be available also to help one to adequately choose the appropriate sequences to fall within the scope of the claims. Conversely, a skilled artisan would know how to design around the invention because the metes and bounds are clearly defined in the claim language and specification. As is well known, the claims must not be read in a vacuum, but in light of the specification and what is well known in the art, and thereby given the broadest interpretation possible, *In re Marosi et al* 218 U.S.P.Q. 289, at 292 (C.A.F.C., 1983). Therefore, the objection to the functional definition of the oligonucleotides in the claims is in error.

These arguments notwithstanding, Applicants' new claims 39-42 should not be submitted to the same rejection since they recite specific oligonucleotides for the first and second primers and/or first capture probes. Thus, the concerns about claiming every possible sequence is not relevant to those claims. Specific oligonucleotides for the third and fourth primers and/or second capture probe are not recited, however, because those are not as critical to the practice of the invention as are the other reagents, as long as all reagents have the properties recited in the claims (i.e., $T_m$, etc.).

Claim 38 has been amended to better define the recited oligonucleotide sequences using Markush language as requested in the Final Rejection.

All of the claims are now believed to meet the requirements of Section 112(2), and the rejection under that portion of the Statute should therefore be withdrawn.

Rejection Under 35 U.S.C. 103

Claims 1-37 have been finally rejected as unpatentable over the combined teaching of Nedjar et al, Brytting et al, Gibbs et al and Findlay et al (WO 90/08840). As far as it applies to claims presently in this rejection, this rejection is respectfully traversed.

As pointed out in Applicants' earlier response, the claims have been amended in several respects which are important to consider in view of the cited art. All original independent claims now recite a limited amount of each primer and high amount of DNA polymerase, which amounts Applicants submit are important for rapid, efficient and simultaneous amplification in their multiplexing invention. Thus, while the matching Tm's are important, as stated in the specification, the amount of primer and DNA polymerase are also very important in order to achieve the desired simultaneous amplification in a rapid fashion (each cycle less than 120 seconds).

Moreover, method Claim 22 recites that each amplification cycle is carried out using the same temperature for primer annealing and primer extension, which temperature is in a narrow range to suit the matched primers used in the method. Thus, the claim more clearly distinguishes the method from routine PCR where one temperature is used for primer annealing and another temperature is used for primer extension. Applicants have found that certain sets of primers, when "matched" as recited in the claims, can be advantageously used to amplify multiple targets simultaneously, as opposed to sequentially, in the presence of specific amounts of primers and DNA polymerase. Applicants' so-called "two-temperature" PCR (one temperature for denaturation, and the second for primer annealing and extension) requires the "matched" primers for multiple target amplification, and such a method is not suggested by the combined art cited in the Final Rejection which teaches "three-temperature" PCR (separate temperatures for denaturation, primer annealing and primer extension).

The rapid cycle (less than 120 seconds) for Applicants' method is highly desirable, and achievable for multiple targets only with the use of the recited amounts of primer and DNA polymerase and the use of "matched" primers which are thereby very efficient under the stringent PCR conditions required for rapidity and efficiency. None of the cited art, alone or in

combination, suggests this critical combination of features needed for rapid and efficient amplification of multiple target nucleic acids.

New Claims 39-42 are more clearly distinguishable over the cited art because they recite specific oligonucleotides for the first and second primers and first capture probe which are not taught or suggested by the cited art. Thus, even if the Final Rejection is maintained for the original claims, Claims 39-42 should be considered free of the prior art.

Upon further review of Nedjar et al, it apparently describes coamplification of HCV and HIV-I DNA, albeit using a "nested" PCR format. However, it does not relate to detection of hCMV DNA. Thus, the characterization of the Final Rejection on page 6 that the "DNA sequence of human CMV is taught in the prior art which has been used by both Nedjar et al and Brytting et al to design primers..." is incorrect. In the first round of the PCR process, primers for HIV-I and HCV DNA are in the reaction mixture. The $T_m$ values for the HIV-I DNA primers are 66 and 65.5°C, and the values for the HCV DNA are 66.5 and 63.1°C. Thus, even if this reference could conceivably teach the coamplification of hCMV DNA and another target, the four primers taught in Nedjar et al do not fall within Applicants' claims. They do not all have the requisite $T_m$ values which would suggest Applicants' invention

Moreover, Nedjar et al teaches a very slow PCR procedure (at least 8 minutes for each cycle, see page 299) compared to Applicants' very rapid (two minutes or less for each cycle) method. Further, Nedjar et al teaches two different primer annealing and extension temperatures (37°C and 72°C) which necessitate very long cycle times since lengthy times are needed for moving from one temperature to another ("ramping" times). Applicants' claims recite a single temperature for both steps. Still further, Nedjar et al teaches the use of merely 2.5 units of DNA polymerase per 100 µl of

solution. This is contrasted with Applicants' claims reciting at least 10 units/100 µl.

Clearly then, Nedjar et al is severely deficient as a primary reference in teaching the presently claimed invention. The question is whether the secondary references provide the missing teaching or any motivation to put the missing pieces together. Applicants submit that they do not.

Brytting et al fails to provide any teaching to overcome the deficiencies of Nedjar et al. Admittedly, it relates to amplification of hCMV DNA as opposed to HCV, but it also teaches "nested" PCR (see pages 129-131) using nested primer pairs. Moreover, different temperatures were used in amplification for primer annealing and primer extension (page 131, first paragraph). Contrary to that teaching, Applicants claim PCR using multiple primer sets simultaneously ("non-nested" PCR) and a single temperature for primer annealing and extension. Moreover, Brytting et al teaches the use of only 1 unit DNA polymerase/50 µl of solution (2 units/100 µl). Admittedly, the Brytting et al cycle was shorter than most (90 seconds), but there is no suggestion that multiple targets can be amplified this quickly in the same reaction mixture. Rather, the "fast" cycles are used in "nested" PCR where primer sets are used in different cycles. Applicants' different invention using "matched" primers, is not suggested by Brytting et al and Nedjar et al together.

Findlay et al admittedly teaches various diagnostic elements or articles having capture probes disposed thereon. However, it fails to overcome the deficiencies noted above in Brytting et al and Nedjar et al. It should be noted also that the existence of Findlay et al is supportive of Applicants' earlier argument that the Section 112(2) rejection of the element claim is in error since the basic structure of such articles is well known. Applicants, however, have provided a novel and patentable improvement by putting

"matched" probes on such articles for use in the rapid PCR method recited in Claim 22.

Gibbs et al is cited for its alleged motivation to simultaneously detect several target nucleic acids using multiple primer sets. This is admittedly more pertinent art that any of Nedjar et al, Brytting et al and Findlay et al. However, it fails to teach or suggest Applicants' claimed invention, by failing to provide the teaching missing from the other cited publications.

Gibbs et al is a detailed analysis of research for finding optimum primer sets for various sequences in the hypoxanthine phosphoribosyltransferase gene. The reference suggests that the desired primers have a critical amount of GC content.

Applicants' claimed method is distinguishable thereover. Applicants method is a "rapid" PCR procedure for amplification of multiple targets wherein each cycle is 120 seconds or less. In order to accomplish this, Applicants require "matched" primers, the same concentration of each primer and a high amount of DNA polymerase.

Gibbs et al describes a process for finding out the optimum amounts of primers needed for detecting multiple sequences on the same gene (see paragraph bridging pages 236-237). Because the multiple targets were detected using gels, they were necessarily of different lengths, and thus different amounts of primers were also necessary. Thus, Gibbs et al teaches that the amounts of each primer set had to be adjusted to compensate for uneven signal strength when they were used in the same reaction mixture (see page 238, paragraph bridging the columns). The amounts of primers ranged from 10 pmol to 25 pmol which is several orders of magnitude less than Applicants' range of from about 0.1 to about 2 μmolar.

In addition, Gibbs et al teaches multiple amplification using only 8 units DNA polymerase/100 μl, and separate temperatures for primer annealing and primer extension (see paragraph bridging pages 236-7). Each

cycle was at least 3 minutes. Detection of the multiple analytes in Gibbs et al was achieved by using conventional gels, not "matched" capture probes as in the present invention.

The PCR of Gibbs et al does not teach Applicants' claimed composition, kit and method whereby considerably more of each primer is used in the reaction mixture. Moreover, Applicants' use of more rapid cycles and the same temperature for primer annealing and primer extension is not suggested by Gibbs et al. Thus, Gibbs et al fails to teach the combined features of Applicants' method which enable rapid and efficient amplification and detection of multiple targets by means of capture probes.

Gibbs et al merely suggests that one can detect multiple targets if one optimizes the amount of each primer used and the targets are of different lengths. Applicants' method is not under such constraints. The amount of primer need not be optimized within the recited range, and the targets can be of any length because capture is not dependent upon resolution on gels. Moreover, Applicants have a more rapid process and have avoided the need for multiple temperatures in each PCR cycle. Only Applicants' combination of "matched" primers, and primer and DNA polymerase amounts make this possible.

The Final Rejection has failed to present a *prima facie* case for obviousness. While some pieces of the claimed invention are shown in the art, the combination of critical features is lacking in actual description and suggestion. No nexus between the cited art and the claimed invention has been pointed out, and it is merely an opinion that one skilled in the art would be able, with reasonable predictability, achieve what Applicants have done, particularly in view of the amendments to the claims. Thus, the rejection should be withdrawn.

Claim 38 appears to be free of the prior art. Thus, with the foregoing amendment to provide more definite language, it should be allowable.

All of the issues presented in the Final Rejection have been addressed, and it believed that the present application in is condition for allowance. If the Examiner has any questions or further amendments to suggest, she is encouraged to call the undersigned so resolution of all issues, and allowance of this application, can be expedited.

Respectfully submitted,

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